

DOSE-RESPONSE EFFECTS OF GAMMA-RADIATION ON SEVERAL GROWTH FUNCTIONS OF *CAMPANULARIA FLEXUOSA*

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Many studies have been done on the effects of ionizing radiation on growth functions of both fresh-water and marine cnidaria. Puckett (1935) suppressed regeneration of hydranth positions in colonies of *Pennaria tiarella* with an x-ray dose of 10,000 roentgens. Daniel and Park (1951, 1953) produced considerable damage to the tentacles of the common brown hydra by x-irradiating the animals at a dose level of 9,600 roentgens. Wermuth and Barnes (1967, 1968) irradiated various portions of five-day-old colonies of *Campanularia flexuosa*. When starter material was irradiated at 81,000 roentgens, lead-shielded new stolons of the same colonies showed an increase in new growth over non-irradiated control colonies.

Brock and Strehler (1963) and Strehler (1964), employing x-ray doses of from 500 to 210,000 roentgens on ten-day-old colonies of *Campanularia flexuosa*, reported an increase in the life-span of the normally cyclically-regressing hydranths of this species with increasing doses of radiation. While these two papers do represent dose-response studies on a single growth function in this species, the data were not presented as dose-response curves. The other papers presented above involve only single-dose studies on the growth functions considered, and therefore cannot be used to produce baseline values for dose effects of ionizing radiation on the growth functions under study.

The present study is concerned with the dose-response effects of ionizing radiation, specifically gamma radiation, on several growth functions in the colonial hydroid *Campanularia flexuosa*. The growth functions monitored post-irradiation for this study include the following: (1) addition of new stolon material; (2) addition of hydranth positions to starters; (3) addition of uprights to stolons; (4) addition of hydranths to stolons post-irradiation; and (5) life-span of hydranths. Observations of certain qualitative phenomena associated with the gamma radiation are also presented. Finally, comparisons of the dose-response effects are noted, leading to some tentative conclusions concerning the nature of determination and differentiation underlying the morphogenetic events associated with these growth functions.

METHODS

Stock colonies of the E strain of *Campanularia flexuosa* were used in these experiments. These organisms were provided by Dr. Sears Crowell, Department of Zoology, Indiana University, Bloomington. Stock colonies, from which starter material for new colonies was obtained, were maintained in a 25-gallon Instant Ocean aquarium. The medium employed was Instant Ocean Synthetic Sea Salts. Both items were supplied by Aquarium Systems, Inc., 33208

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Lakeland Boulevard, East Lake, Ohio, 44094. Approximately 9 liters of Instant Ocean were removed once each week from the aquarium, and replaced with an equal amount of freshly prepared Instant Ocean.

Stock colonies were fed twice daily to build up sufficient biomass for providing starters for new colonies, and irregularly after that, but always several times each week. Experimental colonies were always fed twice daily, morning and evening, during the course of an experiment. In both cases, the food consisted of freshly hatched nauplii of *Artemia salina* (Metaframe San Francisco Bay Brand, obtainable from Wards Scientific Supply, Rochester, New York) filtered, washed once with fresh Instant Ocean, and resuspended after a second filtering in fresh Instant Ocean. New colonies were propagated according to the method of Crowell (1953); *i.e.*, new colonies were initiated by removing an upright, consisting of a stem and associated hydranths, from a well-established stock colony. The upright was then placed under a cotton thread previously tied the short way around an ordinary microscope slide. [See also Wermuth and Barnes (1969) for a diagram of this technique.] In practice, a stock colony is kept in one petri dish, and two already-marked and -tied slides are kept in another petri dish. All manipulations are done under a dissecting microscope. Upright removal is easily accomplished by using a small, chromed, curved surgical scissors. The curvature allows the investigator to see the upright being cut, without at the same time having an uncomfortable or awkward hold on the scissors. Further, the slightly opened legs of the curved scissors can form a small basket for catching the detached upright and moving it through the air-water interface to the second petri dish.

New colonies were started three to a slide. Five slides were placed in one 10-space standard staining rack. Each group of 15 new colonies were referred to as a series for record-keeping considerations. The staining racks were kept either in the Instant Ocean aquarium at a constant temperature (18° C) or in a walk-in cold room at 9° C. Temperatures were kept at a constant value throughout any particular experiment. While in the cold room, the staining racks containing the slides with growing colonies were immersed in staining dishes containing approximately 200 ml of Instant Ocean. The covered staining dishes were kept on a bacteriological shaker table modified to hold them; consequently, the colonies were subject to a gentle oscillation throughout the course of an experiment. For feeding purposes the staining racks were transferred one-by-one to a staining dish on the shaker table containing a thick suspension of brine shrimp nauplii for timed intervals of 3 minutes after which the staining rack was transferred back to its original staining dish. To minimize the effects of decaying egestate and metabolic end-products, the Instant Ocean medium in each staining dish was changed throughout the course of any experiment every two days; that is, at approximately 48-hour intervals.

Gamma radiation was delivered from a source consisting of one dozen rods of Co⁶⁰ which could be raised or lowered from a well located in the center of a room approximately 2m by 2m. For purposes of irradiation, staining dishes containing colonies were placed at set distances from the edge of the well. The five slides were moved to the five staining rack slots closer to the radiation source, so that colonies were oriented with their long axes at right angles to the radiation. In other words, if an imaginary line were drawn from each end of a colony-

containing slide to the source, a triangle rather than a straight line would result. Bunching the five colony-bearing slides at one end of a staining dish accomplished the following: (1) maintained the dose level of the slide furthest from the source as close as possible to that of the slide closest to the source, and (2) approximated the diameter of the vial containing the dosimetry reagent. Dosimetry was accomplished by placing a vial of HE-6 reagent along side of or on top of the staining dish at the level of the slides. The HE-6 dosimetry system is described by McLaughlin, Hussmann, Eisenlohr, and Chalkley (1971).

During the course of the irradiation, which in all cases was 80 minutes, control colonies were kept in a control room adjoining the source room. The temperature of the Instant Ocean medium in the staining dishes containing the colonies being irradiated rose no more than 3° C during the irradiation period, control colony Instant Ocean rose no more than 2° C during the same period. Immediately upon cessation of irradiation, the irradiated medium was discarded and fresh Instant Ocean was added to each staining dish.

Daily records of the growth functions mentioned above were kept for all experimental colonies. Daily records of the developmental condition of individual hydranths were also maintained.

RESULTS

Qualitatively, no immediate difference in the behavior or the appearance on irradiated and non-irradiated colonies of *Campanularia flexuosa* was observed. Hydranths continued to feed; stolons continued their outward growth. At 24 hours post-irradiation, changes in stolon growth and hydranth production by those colonies which had received 80 Krad were observed. Rarely did the starters of these colonies add more than one new hydranth position; in rare cases only did this hydranth position proceed beyond an obviously damaged pedicel stage. In those cases where a hydranth did proceed beyond this point, a small, skinny-looking hydranth with reduced numbers and sizes of tentacles was produced. These hydranths did possess the ability to capture brine shrimp nauplii, indicating the presence of nematocytes in their truncated tentacles. Hydranths which are in developmental stages beyond the pedicel will continue to differentiate into properly formed and functional hydranths which eventually regress, but new hydranths are not regenerated from these positions.

Several cases of disruption of hydranth polarity by gamma radiation were observed. Normally, the long axis of the first hydranth of an upright develops at an obtuse angle relative to the long axis of the distal stolon; the long axis of the second hydranth emerges at a 90° angle to the long axis of the first, with subsequent hydranths emerging in alternate fashion, each one slightly farther away from the stolon; *i.e.*, higher up the upright. In a few cases it was observed that in new uprights which appeared post-irradiation, the first hydranth position emerged with its long axis directed away from the starter. In one case, two hydranth positions emerged at the same distance from the stolon, but 180° from each other on the upright.

Very high radiation levels (80 Krad) almost completely suppressed upright formation on stolons, but did not completely suppress stolon growth or extension itself. This combination of phenomena produced abnormally long stretches of distal stolon, *i.e.*, stolon beyond the last upright. Gamma-irradiated stolons never

showed the "denting" of the coenosarc described by Wermuth and Barnes (1969) when colonies of *Campanularia flexuosa* were x-irradiated. On the other hand, the squaring or blunting of the normally-rounded stolon tip was observed in *Campanularia flexuosa* after exposure to both gamma radiation and x-irradiation.

Quantitative results of the dose-response studies are presented in the following tables and figures. These data are derived from two separate experiments conducted at two temperatures, 9° C and 18° C. The 9° C-series was maintained in individual staining dishes in a cold room; the 18° C-series was maintained in an Instant Ocean aquarium. The 9° C-series received gamma-radiation doses of 0, 16, 24, 40, and 80 Krad. Those of the 18° C series received gamma-radiation doses of 0, 9, 18, 30 and 80 Krad. The difference in dosage between the two experiments was the result of an unannounced change in the configuration of the source. The distance from the source, as measured from a set point, ranged from 8 through 38 cm in intervals of ten cm measured from the set point to the middle of the 5 slides in any one staining dish. Tables I and II are summaries

TABLE I

Summary of dose-response data for several growth function of gamma-irradiated colonies of *Campanularia flexuosa*. Colonies were maintained at 9° C; irradiation, day 11 after starting colonies; experiment terminated on day 19.

| Growth Function | Dose Krad | Response \pm S.D. | Number |
|-----------------------------------------------------------------------------|-----------|---------------------|--------------|
| New stolon material added days 1-8 after irradiation | 0 | Avg. mm of stolon | of colonies |
| | 16 | 34.5 \pm 6.2 | 12 |
| | 24 | 25.5 \pm 7.9 | 13 |
| | 40 | 20.4 \pm 5.1 | 11 |
| | 40 | 13.4 \pm 3.6 | 12 |
| | 80 | 10.8 \pm 4.1 | 13 |
| Hydranth positions added to starters days 1-8 after irradiation | 0 | Avg. no. added | of colonies |
| | 16 | 14.7 \pm 4.9 | 15 |
| | 24 | 8.6 \pm 3.6 | 14 |
| | 40 | 5.5 \pm 2.4 | 13 |
| | 40 | 3.3 \pm 1.4 | 15 |
| | 80 | 0.9 \pm 0.6 | 15 |
| Uprights added to stolons days 1-8 after irradiation | 0 | Avg. no. added | of colonies |
| | 16 | 4.0 \pm 0.6 | 12 |
| | 24 | 3.2 \pm 0.7 | 13 |
| | 24 | 2.0 \pm 0.7 | 11 |
| | 40 | 1.0 \pm 0.5 | 13 |
| | 80 | 0.1 \pm 0.2 | 14 |
| Hydranth positions added to uprights on stolons, days 1-8 after irradiation | 0 | Avg. no. added | of colonies |
| | 16 | 13.2 \pm 2.2 | 12 |
| | 24 | 9.8 \pm 3.4 | 13 |
| | 24 | 3.8 \pm 1.6 | 11 |
| | 40 | 2.0 \pm 1.0 | 13 |
| | 80 | 0.4 \pm 0.5 | 14 |
| Average life span, hydranths on new stolons | 0 | Days | of hydranths |
| | 16 | 17.7 \pm 1.3 | 32 |
| | 24 | 17.4 \pm 0.9 | 52 |
| | 24 | 19.7 \pm 2.3 | 46 |
| | 40 | 21.5 \pm 2.1 | 45 |
| | 80 | 25.0 \pm 2.9 | 29 |

TABLE II

Summary of dose-response data for several growth functions of gamma-irradiated colonies of *Campanularia flexuosa*. Colonies were maintained at 18° C; irradiation, day 9 after starting colonies; experiment terminated on day 14.

| Growth function | Dose Krad | Response ± S.D. | Number |
|-----------------------------------------------------------------------------|-----------|---------------------------------|---------------------|
| New stolon material added days 1-5 after irradiation | 0 | Ave. mm of stolon 14.0 ± 2.8 | of colonies 8 |
| | 9 | 12.1 ± 1.4 | 11 |
| | 18 | 5.8 ± 1.4 | 10 |
| | 30 | 4.1 ± 1.4 | 7 |
| | 80 | 4.5 ± 1.3 | 8 |
| Hydranth positions added to starters days 1-5 after irradiation | 0 | Ave. no. added 8.1 ± 4.4 | of colonies 8 |
| | 9 | 4.9 ± 2.3 | 11 |
| | 18 | 1.4 ± 1.1 | 10 |
| | 30 | 1.7 ± 1.7 | 7 |
| | 80 | 0.1 ± 0.3 | 8 |
| Uprights added to stolons days 1-5 after irradiation | 0 | Ave. no. added 4.9 ± 0.9 | of colonies 8 |
| | 9 | 4.1 ± 0.8 | 11 |
| | 18 | 1.6 ± 0.5 | 10 |
| | 30 | 1.0 ± 0.8 | 7 |
| | 80 | 1.1 ± 0.3 | 8 |
| Hydranth positions added to uprights on stolons, days 1-5 after irradiation | 0 | Ave. no. added 21.8 ± 6.4 | of colonies 8 |
| | 9 | 11.6 ± 3.5 | 11 |
| | 18 | 3.8 ± 1.9 | 10 |
| | 30 | 1.3 ± 1.3 | 7 |
| | 80 | 1.4 ± 0.7 | 8 |
| Average life span of hydranths on new stolons | 0 | Days 7.2 ± 0.8 | of hydranths 60 |
| | 9 | 7.4 ± 1.1 | 87 |
| | 18 | 8.1 ± 1.3 | 74 |
| | 30 | 8.4 ± 1.2 | 33 |
| | 80 | 8.9 ± 2.0 | 42 |
| Average life span of hydranths on starters | 0 | Days 7.1 ± 1.0 | of hydranths 191 |
| | 9 | 7.5 ± 1.0 | 210 |
| | 18 | 8.0 ± 1.2 | 228 |
| | 30 | 8.3 ± 1.0 | 190 |
| | 80 | 8.4 ± 1.5 | 180 |

of the dose-response data for several growth functions of gamma-irradiated colonies of *Campanularia flexuosa* from the two separate experiments described above. They contain information concerning the number of colonies involved in each study, and the number of hydranths monitored for their longevity. Only hydranths which had already appeared by the day of irradiation were included in these studies. Data for the life span of hydranths on starters of the 9° C are not available. Note that the experiment for the 9° C colonies terminated 8 days after irradiation; for the 18° C colonies, 5 days after irradiation. Thus, 9° C colonies were irradiated on day 11 after starting of colonies; 18° C colonies were irradiated on day 9 after starting of colonies. Starting day was day 0.

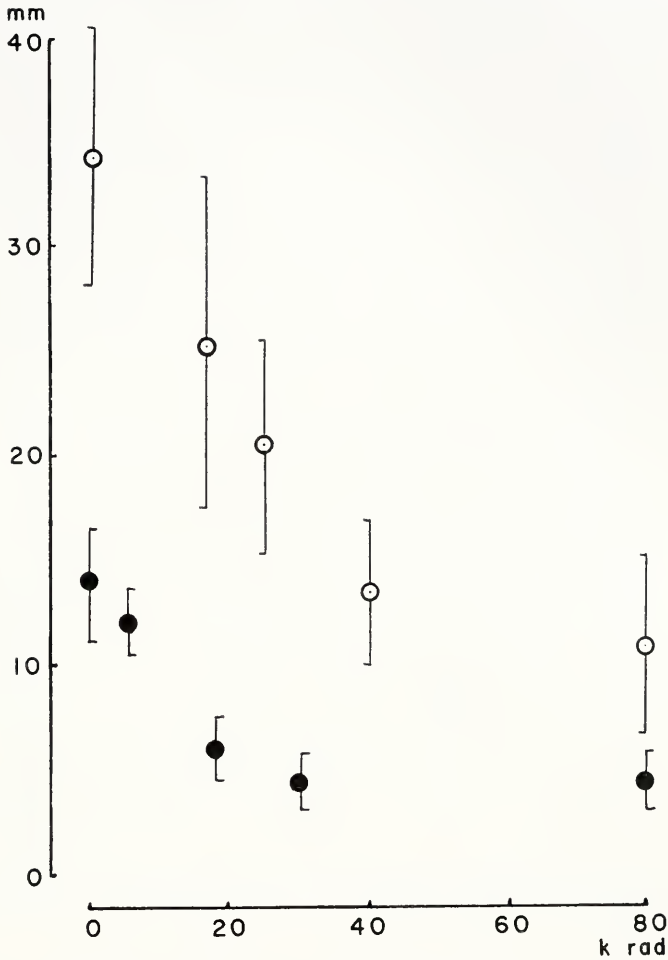


FIGURE 1. Average amount of new stolon material in mm added post-irradiation as a function of gamma-radiation dose in Krad. Open circles are 9° C colonies; closed circles are 18° C colonies. Vertical bars are one standard deviation above and below means.

Figures 1 through 6 present graphically the data presented in the summary tables. Figure 1 presents data on the average amount of new stolon material, in mm of linear growth as measured with the aid of an eyepiece micrometer. With increasing radiation dose, there is a linear decrease in the average amount of linear stolon growth, at least over the dose range from 0 to 30 Krad. An inflection in the curve is noted at that point, especially in the 18° C colonies, so that there is little or no difference in the response of those colonies which received 30 Krad and those that received 80 Krad. The inflection in the curve for the 9° C colonies is less evident but still obvious. A possible explanation of this difference will be presented later.

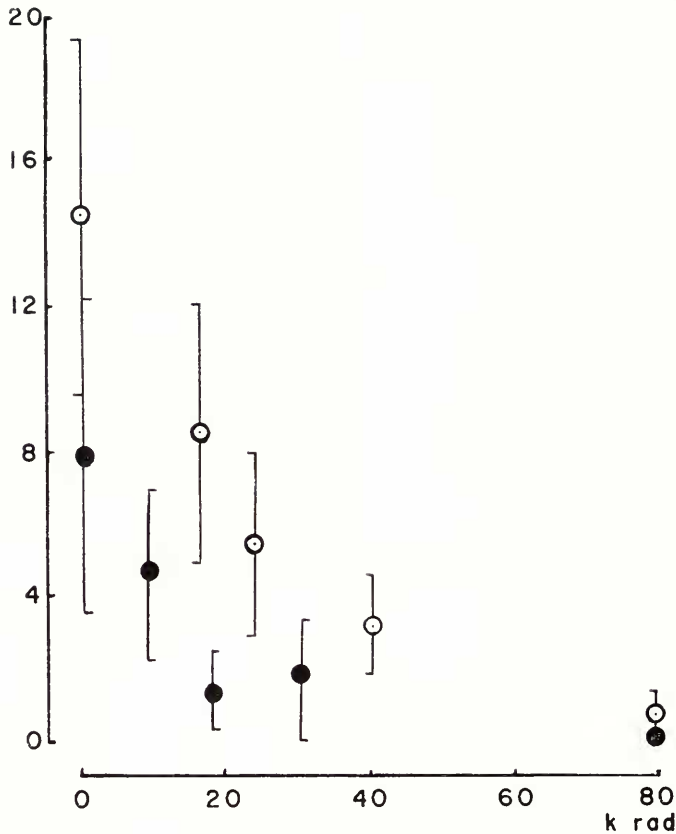


FIGURE 2. Average number of new hydranths added to colony starting material post-irradiation as a function of gamma-radiation dose. Open circles are 9° C colonies; closed circles are 18° C colonies. Vertical bars are one standard deviation above and below means.

In Figure 2, data concerning the number of new hydranth positions added to starters relative to the amount of gamma radiation received is presented. In both cases there is a decreasing number of hydranth positions produced with an increasing level of gamma radiation. The cause of the upward inflection of the 18° C-colony curve at 30 Krad is not known. In any event there is little to distinguish the average number of hydranths produced by starters which received 18 Krad and those that received 30 Krad of gamma radiation. In 18° C colonies, 80 Krad essentially stops hydranth production. The point at 80 Krad represents one new hydranth position generated by eight colonies in the five day period after irradiation.

Figure 3 presents data on the number of new uprights produced by new stolons after gamma radiation. There is a decrease in the number of new uprights produced with increasing dose of gamma radiation over the entire range from 0 through 80 Krad for the 9° C colonies; and a decrease in the number of new uprights produced with increasing dose over the range from 0 to 30 Krad for the

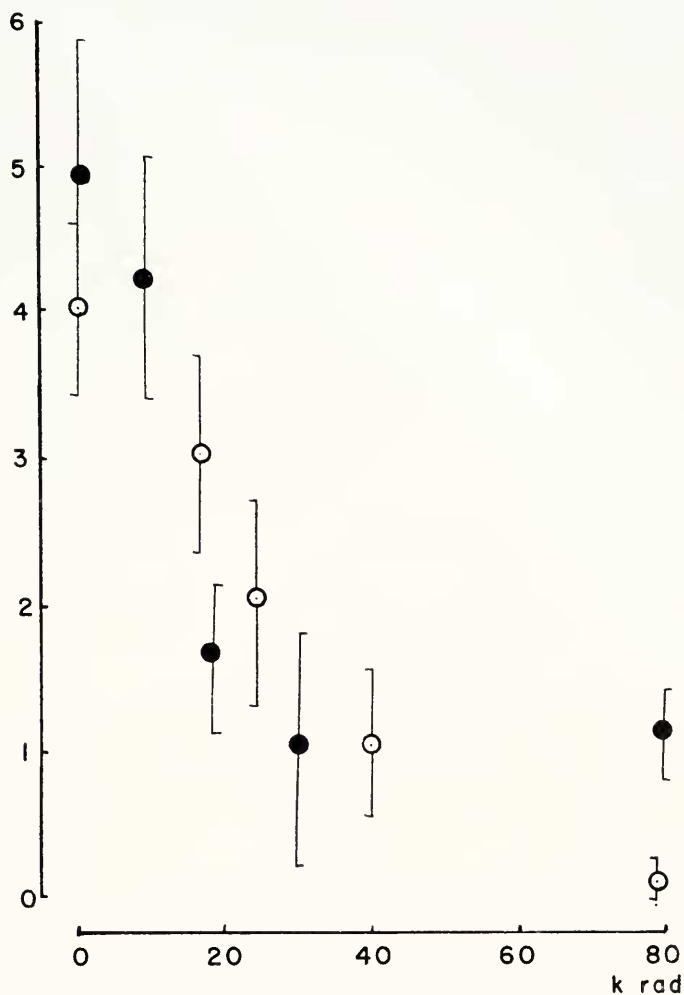


FIGURE 3. Average number of new uprights added to new stolons post-irradiation as a function of gamma-radiation dose in Krad. Open circles are 9° C colonies; closed circles are 18° C colonies.

18° C colonies, with little difference in the number of new uprights produced by 18° C colonies which had received 30 Krad, and those colonies which had received 80 Krad of gamma radiation. A possible explanation of this difference between 9° C and 18° C colonies will be presented in the discussion.

In Figure 4, data are presented on the number of hydranth positions added to uprights on stolons of 9° C and 18° C colonies receiving gamma radiation doses of 0 through 80 Krad. These curves are quite similar to those presented in Figure 3, there being a decrease in hydranth production with increasing radiation dose over the entire experimental range of 0 through 80 Krad for 9° C colonies; and a decrease in hydranth production with increasing radiation dose over the range

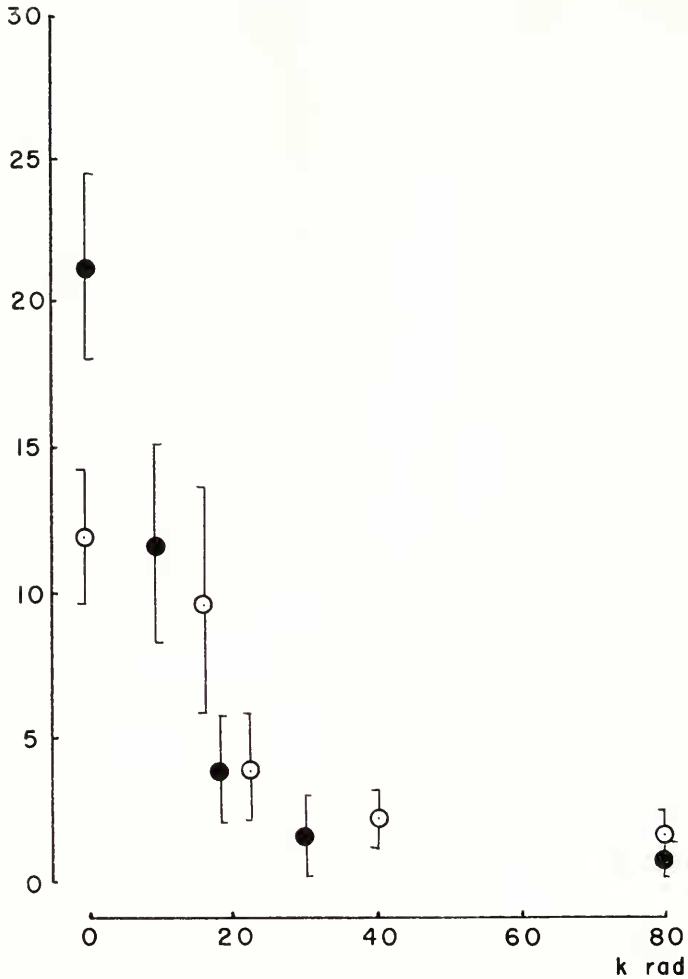


FIGURE 4. Average number of hydranths added to uprights of new stolons post-irradiation as a function of gamma-radiation dose in Krads. Open circles are 9° C colonies; closed circles are 18° C colonies. Vertical bars are one standard deviation above and below means.

from 0 through 80 Krads for 18° C colonies with little difference in hydranth production between 18° C colonies which received 30 Krads, and 18° C colonies which received 80 Krads of gamma radiation. Again, a possible explanation for the inflection in the curve of 18° C colonies for this growth function is presented in the discussion section of this paper.

Figures 5 and 6 present data on the average life span of new hydranths on new stolons (Fig. 5) of 9° C colonies which received from 0 through 80 Krads of gamma radiation; and on the average life span of hydranths on both new stolons and starters of 18° C colonies which received from 0 through 80 Krads of gamma radiation (Fig. 6). In all cases there is an increase in hydranth life span with

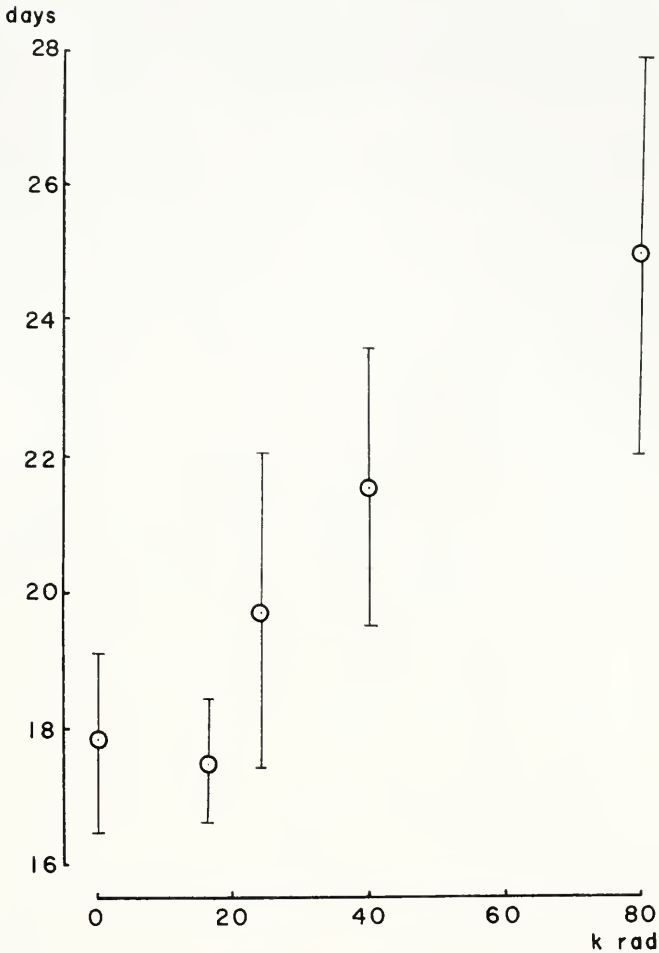


FIGURE 5. Average life span of new hydranths on new stolons of 9° C colonies as a function of gamma-radiation dose in Krad. Vertical bars are one standard deviation above and below means.

an increase in radiation dose. These data corroborate the data presented by Strehler (1964) and Brock and Strehler (1963) for x-irradiated hydranths of *Campanularia flexuosa*. While the curve is essentially linear over the range of 0 through 80 Krad for hydranths of 9° C colonies, there is an inflection in the curve of 18° C colonies at 30 Krad. There is little difference between the response of hydranths on starters and hydranths on stolons to gamma radiation over the range of 0 through 80 Krad in 18° C colonies.

DISCUSSION

Altman, Gerber, and Okada (1970) have stated that the primary radiation damage consists of damage to the cellular DNA itself. Employing this state-

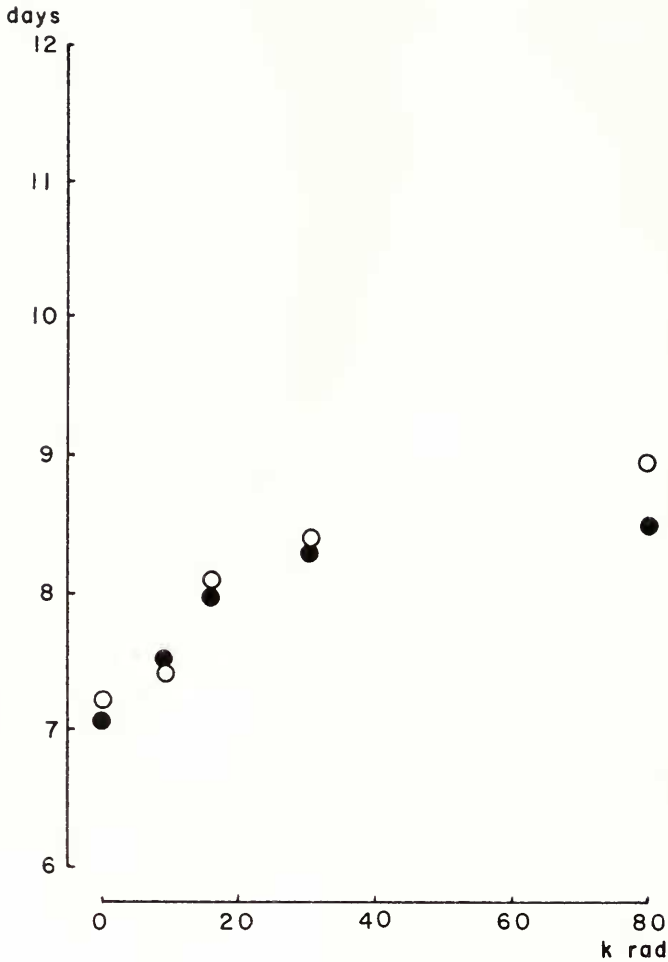


FIGURE 6. Average life span of hydranths on starters and new stolons of 18° C colonies as a function of gamma-radiation dose in Krad. Open circles are average life spans of hydranths on new stolons; closed circles are average life spans of hydranths on starters.

ment as a starting point, the data presented in the tables and figures are easily accounted for with only minor problems presented by the difference in temperature between the two experiments. If the primary radiation effect is damage to cellular DNA, and if the cellular DNA of *Campanularia flexuosa* functions as it does in other organisms, then it is not surprising to find that increasing doses of gamma radiation decreases the four growth functions of: (1) new stolon material produced, (2) new hydranth positions added to starters, (3) new uprights added to stolons, and (4) new hydranth positions added to uprights on stolons. All of these growth functions presumably depend upon the production of new cells. The greater the amount of radiation received by the organism, the greater is the chance that any

particular portion of DNA will be disrupted by the ionizing radiation. Because of both aborted mitoses and the failure to manufacture proper messenger RNA molecules from the disrupted DNA, the production of new cells that would be involved in stolon growth (as stolon extension), hydranth production, and upright production would be less.

As a general rule, the higher the ambient temperature around colonies of *Campanularia flexuosa*, the greater is the rate of stolon growth, hydranth production, and upright production. Consequently any developmental period is shortened in time presumably including the time during which a particular structure is being determined. In slower growing colonies, *i.e.*, those grown at lower temperatures, there is a greater probability of a structure being covertly determined, but not yet morphologically distinct; that is, the period of covert determination is longer in slower-growing colonies than it is in faster-growing colonies. Ionizing radiation does not seem to interfere with subsequent differentiation after determination of that structure has occurred. If one applies this criterion to hydranth development, it would seem that hydranth development involves at least two separate determinations. At gamma radiation levels of 30 through 80 Krad, at least, new hydranth positions do appear but these new positions do not proceed past the pedicel stage of development except in rare cases. Those hydranths that have progressed to the cone stage of development at the time of irradiation proceed through the remaining developmental sequence, become normal-appearing feeding hydranths, and eventually regress, though they do not regenerate. Crowell (1974) reported a similar critical period of hydranth determination for dissociated hydranths, and actinomycin-D-treated hydranths of *Campanularia flexuosa*, as did Belousov, Badenko, Katchurin, and Kurilo (1972) for both cyanide-treated and dinitrophenol-treated hydranths of this species.

Since in colonies grown at higher temperatures, the period of covert determination without morphological appearance is shorter than in colonies grown at lower temperatures, it would not be surprising to find, if the value of 30–40 Krad is accepted as the maximum dose needed to suppress determination, that a gamma radiation dose of 80 Krad would yield little or no greater effect in faster growing colonies with few structures in covert determination, but would cause an apparently greater effect in slower-growing colonies in which the period of covert determination is much longer. The lack of any effect on 18° C colonies dosed with 80 Krad over those dosed with 30 Krad will appear as an inflection at 30 Krad in the dose-response curves of those growth functions so considered. Likewise, the apparent effect in 9° C colonies at both 40 Krad and 80 Krad will be no inflection in these dose-response curves. It is suggested that if colonies were carefully controlled as to ambient temperature over the range of 9° C through 18° C in small steps of perhaps 1° C, and then if such colonies were gamma-irradiated at a dose level of 80 Krad, an estimate of the time required for the determination of hydranth positions could be made.

This work was performed while the first author was a National Cancer Institute Special Research Fellow. During his tenure, he was also a visiting assistant professor in the Department of Life Sciences, Indiana State University, Terre

Haute, Indiana. He wishes to thank Dr. Charles D. Barnes of that department for his aid and sponsorship. This work was performed under fellowship 1 FO3 C14 53590-01 from the National Institutes of Health.

SUMMARY

1. Colonies of *Campanularia flexuosa* were gamma-irradiated with doses ranging from 0 through 80 Krad. Several growth functions were monitored. These growth functions include hydranth production by starters and stolons, upright production by stolons, stolon growth, and longevity of hydranths on both starters and stolons.

2. All growth functions show a decrease with increasing doses of gamma radiation, except hydranth longevity. Hydranth longevity increases with increasing dose.

3. A difference in temperature yields differences in the growth-response curves of these growth functions.

4. The implications of the dose-response curves are discussed in terms of the hypothesis that the primary effect of ionizing radiation is damage to DNA molecules.

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